Cell, Vol. 88, 811–821, March 21, 1997, Copyright ©1997 by Cell Press

# Neural Targeting of Mycobacterium leprae Mediated by the G Domain of the Laminin-α2 Chain

**Anura Rambukkana,* James L. Salzer,† Peter D. Yurchenco,‡ and Elaine I. Tuomanen***
*Laboratory of Molecular Infectious Diseases
The Rockefeller University
New York, New York 10021
†Departments of Cell Biology, Neurology and the Kaplan Cancer Center
New York University Medical Center
New York, New York 10016
‡Department of Pathology
Robert Wood Johnson Medical School
Piscataway, New Jersey 08854

## Summary

**We report that the molecular basis of the neural tropism of Mycobacterium leprae is attributable to the specific binding of M. leprae to the laminin-α2 (LN-α2) chain on Schwann cell–axon units. Using recombinant fragments of LN-α2 (rLN-α2), the M. leprae-binding site was localized to the G domain. rLN-α2G mediated M. leprae binding to cell lines and to sciatic nerves of dystrophic *dy/dy* mice lacking LN-α2, but expressing laminin receptors. Anti-$\beta_4$ integrin antibody attenuated rLN-α2G-mediated M. leprae adherence, suggesting that M. leprae interacts with cells by binding to $\beta_4$ integrin via an LN-α2G bridge. Our results indicate a novel role for the G domain of LN-2 in infection and reveal a model in which a host-derived bridging molecule determines nerve tropism of a pathogen.**

## Introduction

Mycobacterium leprae, the causitive organism for leprosy, is unique among bacterial pathogens in its ability to invade the peripheral nervous system. The neural tropism of this disease has been recognized for almost 150 years (Danielssen et al., 1848) but has remained unexplained. At present, it is estimated that 2–3 million leprosy patients in the world are physically disabled as a result of damage to peripheral nerves and the attendant sensorimotor loss (Job, 1989; Noordeen et al., 1992).

Clinical and histopathological evidence indicates that the Schwann cell of the peripheral nerve is the principal target of M. leprae (reviewed in Lumsden, 1959; Mukherjee and Antia, 1986). Schwann cells are unable to destroy the pathogens that reside intracellularly, and access of therapeutic agents to this site is limited owing to the blood–nerve barrier. Although long-term multidrug treatment can achieve bacteriological cure, it does not reverse nerve-function loss in leprosy patients. Therefore, development of therapeutic strategies that interrupt the M. leprae–Schwann cell interaction will be important for the prevention of nerve damage.

The specificity of a bacterial–host cell interaction and the pattern of tissue distribution of host cell receptors determine which tissues are ultimately infected by a pathogen. Thus, a specific Schwann cell–M. leprae affinity is likely to underlie the unique neural tropism of the disease. Although in vitro nerve tissue cultures recapitulate the neural tropism of M. leprae (Lumsden, 1959; Mukherjee and Antia, 1986), virtually nothing is known about the molecular basis of this neural affinity. In this study, we establish the molecules that mediate this bacterial–host cell interaction.

Pathogenic bacteria attach to their preferred host target by molecules on the bacterial surface, termed adhesins, that recognize cognate host-cell receptors (Falkow, 1991; Tuomanen et al., 1995). This attachment can be achieved by direct binding or indirectly by adsorbing a bridging ligand of host origin onto the bacterial surface, which then enables the pathogen to "tag along" a natural pathway determined by bridging ligand–host receptor interactions (Hoepelmann and Tuomanen, 1992). In this regard, the integrin family of host-adhesion molecules that mediate cell–cell and cell–extracellular matrix contacts have gained much attention as cognate receptors for bacterial adhesion and entry (Isberg and Leong, 1990; Relman et al., 1990).

In the endoneurium of peripheral nerve, all Schwann cells are covered by basal lamina, a characteristic feature that distinguishes Schwann cells from fibroblasts and macrophages (Gamble and Eames, 1964). Since M. leprae must interact with the basal lamina in order to reach the Schwann cell, tropism to this site and perhaps cellular entry might involve the components of Schwann cell basal lamina. Schwann cell basal lamina is comprised of laminin, type IV collagen, entactin/nidogen, and heparin sulfate proteoglycans (Cornbrooks et al., 1983; Jaakkola et al., 1989; Sanes et al., 1990). Although there is evidence that M. leprae binds to fibronectin (Schorey et al., 1995), this binding may not be relevant for M. leprae interactions with Schwann cells in vivo, since both fibronectin mRNA and protein are absent in Schwann cell basal lamina in situ and in primary cultures (Cornbrooks et al., 1983; Jaakkola et al., 1989). Conversely, considering the continuous presence of laminin around the Schwann cell–axon unit in vivo (Cornbrooks et al., 1983; Jaakkola et al., 1993), laminin appeared to be a good candidate as an initial target for M. leprae.

Laminins (LNs) are glycoproteins comprised of three polypeptide chains, α, β, and γ. The α chain distinguishes itself by having an extra domain at the C-terminus, i.e., the G domain (see Figure 5A) (Burgeson et al., 1994; Timpl and Brown, 1994). At least 10 genetically distinct LN chains have been identified (α1, α2, α3, α4, α5, β1, β2, β3, γ1, and γ2), which assemble into 11 different LN isoforms (LN-1 to -11), each with restricted tissue distribution (reviewed in Timpl and Brown, 1994; Engvall and Wewer, 1996). In the Schwann cell basal lamina, the predominant LN variant is LN-2 (merosin), which is composed of the tissue-specific α2 heavy chain together with the β1 and γ1 light chains (see Figure 5A) (Leivo and Engvall, 1988; Engvall et al., 1990, 1993). LN-2 not only forms a major basement membrane network, but also enables a variety of functions of neural cells (Engvall et al., 1992; Yurchenco et al., 1992; Anton et al., 1994).

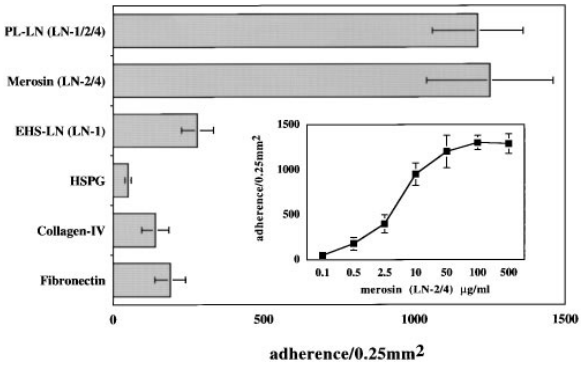


Figure 1. M. leprae Binds Preferentially to LN-2/4

Quantification of M. leprae adherence to immobilized ECM proteins. M. leprae ($5 \times 10^8$) was overlaid onto 0.1 μg of ECM proteins. Number of adherent bacteria within 0.25 $mm^2$ grid area of each well was quantified after 60 min of incubation, and the data were expressed as the mean ± SD values from 4–5 wells. Inset: binding of M. leprae ($5 \times 10^8$) to LN-2/4 with increasing concentration. Each experiment was performed at least 3 times with similar results.

The major cell receptors for LN are the members of the integrin superfamily (Hynes, 1992; Timpl and Brown, 1994; Mercurio, 1995). Several integrin receptors bind to LN (Mercurio, 1995), and $\alpha_6\beta_1$ and $\alpha_6\beta_4$ integrins, particularly the $\beta_4$ subunit, appear to be involved in the Schwann cell interaction with LN (Einheber et al., 1993; Jaakkola et al., 1993; Feltri et al., 1994; Niessen et al., 1994).

In this study, we provide evidence that the neural tropism of M. leprae involves the bacterial binding to the G domain of the LN-α2 chain, which serves as a bridge between M. leprae and the native LN receptors on Schwann cells. We identify the LN-α2G domain as both the bacterial and human cell binding site. We suggest the $\beta_4$ integrin subunit may serve as host-cell receptor for LN-α2G-mediated M. leprae–cell interaction. This study presents a remarkable example of tissue-specific tropism of a bacterial pathogen determined by a host-derived bridging molecule and a description at the molecular level for targeting of a bacterial pathogen to the nervous system.

## Results

### M. leprae Binds Preferentially to LN-2/4

To identify which extracellular matrix (ECM) protein(s) on Schwann cell basal lamina might serve as a preferential target of M. leprae, the adherence of M. leprae to immobilized ECM proteins was quantified using a solid-phase bacterial-adherence assay. Using 0.1 μg of immobilized ECM proteins, it was found that M. leprae ($5 \times 10^8$) bound preferentially to merosin (LN-2 and LN-4) and placental LN (LN-1, -2, and -4), as compared to Engelbreth-Holm Swarm (EHS) LN (LN-1), type IV collagen, heparin sulphate proteoglycan, and fibronectin ($p < 0.003$) (Figure 1). Specificity and saturable binding of M. leprae to LN 2/4 was demonstrated by the concentration-, dose-, and time-dependent adhesion kinetics (Figure 1, inset; data not shown). Although M. leprae bound to high concentrations of LN-1 and fibronectin, saturation required concentrations (1000 μg/ml) 10-fold higher than for LN-2/4. M. leprae isolated from four different armadillos showed similar preferential binding to LN-2/4, as compared to other matrix proteins (data not shown). Denaturation of LN-2/4 by heat treatment decreased the binding by 73% (from 1200 ± 125 to 220 ± 38 bacteria), suggesting that M. leprae binding to LN-2/4 is dependent on the conformation of the laminin molecules. Since M. leprae preferentially bind to LN-2/4, and the difference between these isoforms is the different assembly forms of individual LN chains, we concluded that adherence of M. leprae may involve an LN chain specific for LN-2/4 isoforms but not the chains shared by LN-1, -2, and -4.

### M. leprae Adherence to Schwann Cells Is Mediated by LN-2

To maintain physiological functions of Schwann cells appropriately (Bunge et al., 1990), a well-characterized in vitro myelinating Schwann cell–neuron coculture system was used, in which LN is expressed on the outer surface of Schwann cell–axon units as it does in vivo (Einheber et al., 1993, 1995). Cocultures grown for 3 months were inoculated with 1–5 × $10^8$ M. leprae, and adherent bacteria were detected by acid-fast labeling. As early as 30 min after inoculation, M. leprae was avidly bound to and aligned along nerve fibers (Figures 2A and 2B). Adherence was dependent on bacterial dose and appeared to increase with the age of the cocultures (4 to 12 weeks). Immunolabeling with antibodies to total LN revealed that the older cultures displayed qualitatively greater LN deposition (data not shown), suggesting that increased binding of M. leprae to older cultures parallels the increased expression of LN on Schwann cells. Preincubation of M. leprae with purified LN-2/4, but not type IV collagen or fibronectin, competitively inhibited M. leprae adherence to Schwann cells. Adherence in the presence of BSA, LN-2/4, type IV collagen, and fibronectin was 575 ± 92, 98 ± 26, 510 ± 78, and 521 ± 67 bacteria per 100 cells, respectively ($p = 0.003$). Preincubation of cocultures with polyclonal antibodies to total placental LN, but not type IV collagen, significantly blocked the binding of M. leprae (data not shown). These results suggest that LN-2/4 plays a significant role in M. leprae adherence to Schwann cell–neuron cultures.

To clarify further which LN isoform mediates the M. leprae–Schwann cell interaction, the individual LN chains expressed in Schwann cell–neuron cultures were identified. Cocultures strongly reacted with antibodies to LN-α2, -β1, and -γ1 chains, but not the α1 and β2 chains (Figures 3A and 3B). The absence of the LN-α1 chain and the expression of the β1 chain in such cultures have been shown previously (Einheber et al., 1995). Since the β2 chain is responsible for the assembly of LN-4 (Timpl and Brown, 1994), the absence of staining for the β2 chain in Schwann cell–neuron cultures (Figure 3A) indicates that LN-4 is absent and that LN-2 is the principal LN isoform in the Schwann cell basal lamina. Therefore, the adhesion of M. leprae to the Schwann cells reflects the activity of LN-2. Figure 3C demonstrates the colocalization of M. leprae and the G domain

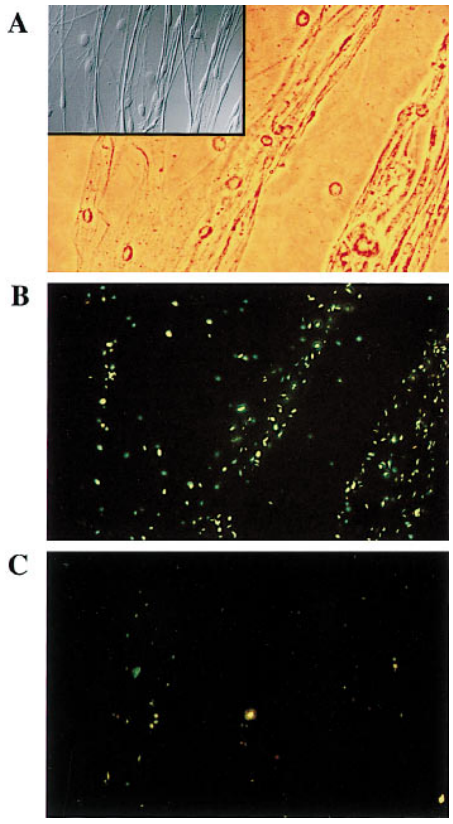


Figure 2. Inhibition of M. leprae Adherence to Schwann Cell–Neuron by LN-2

Myelinating Schwann cell–neuron coculture grown for 8 weeks were inoculated with M. leprae, and adherent bacilli were detected by auramine-rhodamine acid-fast labeling.

(A) Phase-contrast image after acid-fast staining. Inset: Nomarski image of intact myelinating Schwann cell culture before acid-fast staining.

(B) Acid-fast labeling of M. leprae corresponding to (A).

(C) Preincubation of M. leprae with merosin–LN-2/4 (200 μg/ml).

of LN-α2 chain on Schwann cell–neuron. Taken together with the ability of M. leprae to distinguish LN-2 from LN-1 (Figure 1), these results suggest that bacteria specifically bind to the α2 chain of LN-2.

To determine if LN-2 could also mediate M. leprae adherence to Schwann cells in the absence of neurons, primary Schwann cells were cultured with forskolin, a drug that elevates cAMP and mimics some of the effects of axonal contact (Jessen and Mirsky, 1991). Binding of M. leprae ($5 \times 10^8$) increased (from 87 ± 22 to 320 ± 70 bacteria per 100 cells; $p = 0.008$) when Schwann cells were treated with forskolin. This binding correlated with increased expression of LN-α2 chain, as compared to untreated Schwann cells (data not shown).

### M. leprae Fails to Adhere to Peripheral Nerves Deficient in LN-α2 Chain In Situ

Dystrophic *dy/dy* mice have a specific defect in the expression of the LN-α2 chain, whereas the expression of γ1 and β1 LN chains or type IV collagen is normal (Sunada et al., 1994; Xu et al., 1994). Using an in situ bacterial-adherence assay (Falk et al., 1993), binding of M. leprae to fresh-frozen sections of normal and homozygous *dy/dy* sciatic nerves was compared. In control nerve sections, M. leprae bound avidly to the endoneurium, which is densely packed with Schwann cells (Figure 4A), and adhesion was inhibited by 75% in the presence of excess (200 μg/ml) LN-2/4 (reduced from 280 ± 55 for control to 65 ± 22 after preincubation with LN 2/4; $p = 0.005$). In experiments with *dy/dy* mice, sections from the distal part of the sciatic nerves were used, since Schwann cells are frequently absent from the proximal spinal nerves (Bradley et al., 1975). Normal presence of Schwann cells was demonstrated by immunolabeling using Schwann cell-specific S-100 antigen; myelination also appeared normal (Figure 4, insets). Binding of M. leprae to the sections of sciatic nerves from *dy/dy* mice was reduced by 85% compared to controls (from 280 ± 55 to 42 ± 11 bacteria; $p = 0.0034$) (Figure 4B). Control FITC-labeled E. coli did not bind to the sciatic nerves of either wild-type or *dy/dy* mice. The lack of adherence of M. leprae to *dy/dy* nerves provides evidence for the use of LN-α2 chain by M. leprae for the interaction with peripheral nerves.

### M. leprae Binds to the G Domain of the LN-α2 Chain

To map the M. leprae-binding site of the LN-α2 chain, two recombinant (r) human cDNA fragments of LN-α2 chain were expressed in a eukaryotic expression system capable of secreting functional glycoproteins. Expressed fragments extended from the N-terminal VI, V, IVb, and the first third of domain IIIb (rLN-α2[VI–IVb]′), and the C-terminal G domain from G1 through G5 subdomains (rLN-α2G). Both rLN-α2G and rLN-α2(VI–IVb)′ were secreted and could be detected in immunoblots with antibodies specific for LN-α2 chain.

Binding of M. leprae to immobilized rLN-α2(VI–IVb)′ and rLN-α2G (Figure 5A, right) was analyzed in the solid-phase bacterial-adhesion assay and compared to rLN-α2G and other proteolytic fragments of LN-1 (Figure 5A, left). M. leprae strongly bound to the C-terminal rLN-α2G fragment, but not to the N-terminal rLN-α2(VI–IVb)′ fragment of the α2 chain (Figure 5B). Also, M. leprae showed no binding to the rLN-α1G or proteolytic fragments of LN-1, including the E3 and E8 fragments of native LN-α1G, even at concentrations as high as 100 μg/ml. Binding was dependent on the concentration of rLN-α2G, reaching saturation at 50 μg/ml (Figure 5B, inset). Preincubation of M. leprae with excess LN-2/4 (200 μg/ml) abolished the binding to rLN-α2G (10 μg/ml) by 84% (from 510 ± 55 to 90 ± 18 bacteria; $p = 0.0007$). These results suggest that the M. leprae-binding site on the laminin-α2 chain resides within the G1–G5 subdomains at the C-terminal end.

rLN-α2G domain binds to heparin, a property exploited in developing a purification protocol (Yurchenco et al., 1993). To determine whether the M. leprae recognition site was the same or different from the heparin-binding site of the G domain, competitive inhibition experiments were performed. Binding of M. leprae to rLN-α2G was not affected in the presence of heparin either in solid-phase or in solution, even at a concentration of 1 mg/ml (325 ± 85 versus 305 ± 72 bacteria).

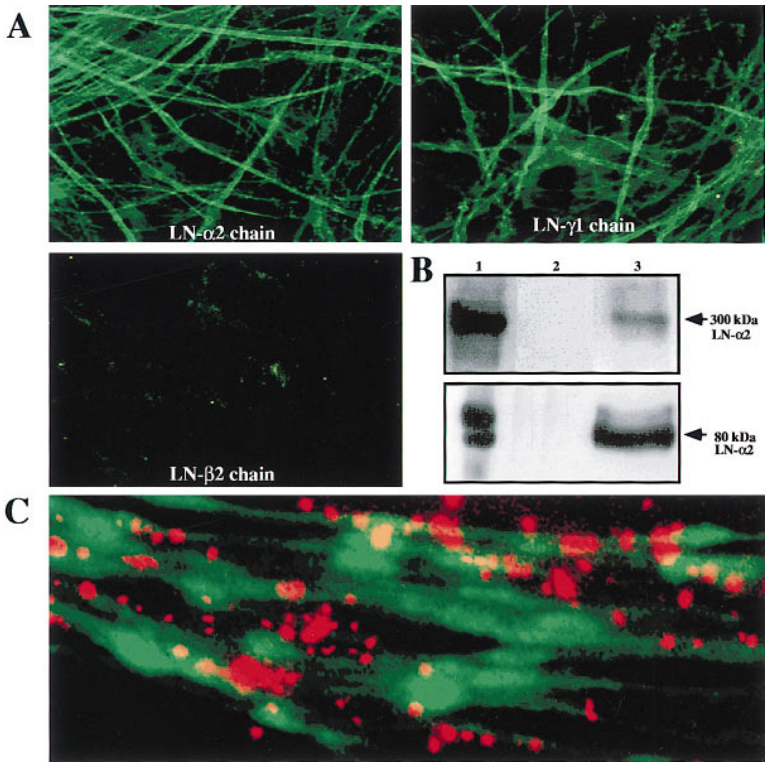


Figure 3. Colocalization of M. leprae and LN-α2G on Myelinated Nerve Fibers

(A) Immunofluorescent micrograph of 8-week-old Schwann cell–neuron cocultures labeled with monoclonal antibodies specific for α2, γ1, and β2 LN chains as indicated.

(B) Immunoblot analysis of LN-2 in total lysates of Schwann cell–neuron cocultures. Schwann cell–neuron extracts (lane 3) were separated by 6% (above) and 10% (below) SDS–PAGE, transferred to PVDF membranes, and immunostained with PAbs to 300 kDa fragment of LN-α2 (above) or rLN-α2G (below). LN-2/4 (lane 1) and EHS LN/LN-1 (lane 2) are shown as positive and negative controls, respectively.

(C) Colocalization of M. leprae and the G domain of LN-α2 chain on myelinating Schwann cell–neuron culture by double immunofluorescence using mouse MAb to native phenolic glycolipid-1 of M. leprae (in red/orange) and PAb to rLN-α2G (in green).

This suggested that the M. leprae-binding site was independent of the heparin-binding site within the α2G domain.

### The G Domain of the LN-α2 Chain Mediates M. leprae Adherence to Schwann Cells In Vitro and In Situ

To demonstrate directly that the LN-α2G domain can mediate M. leprae adherence, rLN-α2G domain was tested for the ability to enhance M. leprae adherence to primary Schwann cells poorly expressing LN-2. Confluent cultures of forskolin-treated primary Schwann cells were inoculated with $1 \times 10^8$ M. leprae in the presence and absence of rLN-α2G domain. M. leprae adherence to Schwann cells was significantly increased in a concentration-dependent fashion after preincubation of bacteria with rLN-α2G domain (Figures 6A–6D). rLN-α2G did not mediate the binding of closely related Mycobacterium tuberculosis to Schwann cell cultures in similar experimental conditions ($<$40 as compared to $>$400 M. leprae per 100 cells). These data suggest that M. leprae–Schwann cell interaction is specifically mediated by the G domain of LN-α2 chain.

The M. leprae-binding activity of the rLN-α2G domain was also apparent in an in situ adherence assay to sections of sciatic nerves from *dy/dy* mice that lack LN-α2 chain. While M. leprae fails to adhere to *dy/dy* nerve in situ (Figure 4B), preincubation of the bacteria with rLN-α2G domain reconstituted adherence to almost 80% of wild-type levels (from 29 ± 12 to 145 ± 38 [$p = 0.009$]; Figure 6F) in a concentration-dependent manner. In contrast, rLN-α2G neither increased binding to control nerve nor mediated M. tuberculosis adherence to *dy/dy* nerves in situ (data not shown). This suggests that exogeneous rLN-α2G domain directly mediates M. leprae adherence to *dy/dy* nerves in situ, most probably using an existing LN-2 receptor(s). In this context, it was found that in situ expression of $\alpha_6\beta_4$ integrin, a major LN receptor of Schwann cells, appears to be normal in *dy/dy* peripheral nerve (Figure 6E).

### Heterologous Model to Identify Host Cell Receptor(s) for LN-α2G-Mediated Interaction of M. leprae: Role of $\beta_4$ Integrin

A major LN-2 receptor on Schwann cells is the integrin $\alpha_6\beta_4$ (Einheber et al., 1993; Jaakkola et al., 1993; Feltri et al., 1994; Niessen et al., 1994). To test the role of $\alpha_6\beta_4$ integrin in LN-mediated M. leprae adherence, a heterologous model using cell lines that do not deposit LN-α2 chain, but express $\alpha_6\beta_4$ integrin, was established. HBL-100 and Cos-7 cells strongly express $\alpha_6\beta_4$ integrin and have little $\alpha_6\beta_1$ (Sonnenberg et al., 1990) (Figure 7A, inset; unpublished data). Erythroleukemic K562 cells were chosen as a negative control because they only express fibronectin receptor $\alpha_5\beta_1$ integrin and lack known LN receptors, including $\alpha_6\beta_4$ (Delwel et al., 1993). None of these cell lines express detectable levels of LN-α2 chain, and the direct binding of M. leprae to these cell lines was also negligible (data not shown). In the presence of LN-2/4, M. leprae adhered to HBL-100 and Cos-7 cells, whereas only a low number of bacteria were bound to K562 cells, regardless of the laminin concentration (Figure 7A). Type IV collagen and fibronectin did not influence the binding of M. leprae to HBL-100 and Cos-7 cells (data not shown). rLN-α2G domain also mediated M. leprae adherence to Cos-7 cells in a concentration-dependent manner (Figure 7B), and this binding

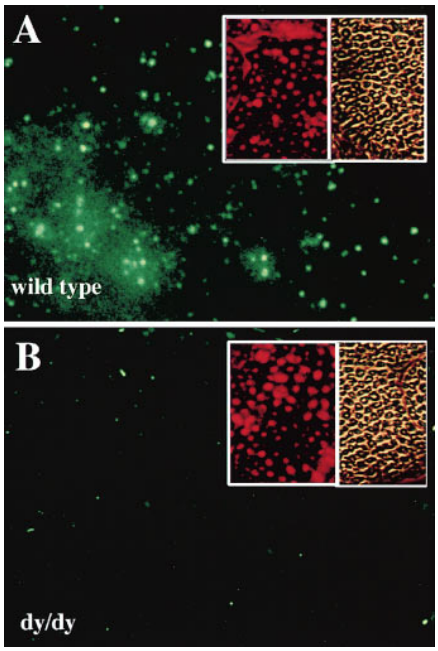


Figure 4. M. leprae Fails to Adhere to LN-α2 Chain-Deficient *dy/dy* Peripheral Nerves In Situ

In situ-adherence of M. leprae to the sciatic nerves from (A) wild-type and (B) *dy/dy* mice. Left insets: staining with antibody to S-100 antigen to demonstrate the densely packed Schwann cells. Right insets: phase-contrast image of myelinated Schwann cells in the endoneurium of both wild-type and *dy/dy* nerves. Experiment was repeated three times from two *dy/dy* and two control mice with similar results.

was more effective as compared to total LN-2/4 (Figures 7A and 7B). In contrast, rLN-α2(VI–IVb)′ or rLN-α1G did not mediate bacterial adherence to Cos-7 cells even at high concentration (100 μg/ml). Binding of $1 \times 10^8$ M. leprae in the presence of 25 μg/ml of rLN-α2G, rLN-α2(VI–IVb)′, and rLN-α1G was 369 ± 19, 19 ± 8, and 10 ± 3 bacteria per 100 cells, respectively ($p = 0.001$). Furthermore, rLN-α2G failed to mediate binding of closely related M. tuberculosis and nonrelated B. subtilis (gram positive) and E. coli (gram negative) to Cos-7 cells (Figure 7B, inset). These data suggest that specific binding of M. leprae to rLN-α2G mediates the bacterial adherence to $\alpha_6\beta_4$-bearing host cells.

To determine whether LN-α2G-mediated M. leprae–Cos-7 cell interaction can be blocked by antibodies to known LN-receptor integrins, confluent cultures of Cos-7 cells were preincubated with previously characterized blocking MAbs against $\beta_1$, $\beta_4$, $\alpha_2$, $\alpha_3$, $\alpha_4$, and $\alpha_6$ integrin subunits, prior to the addition of M. leprae ($1 \times 10^8$) treated with 25 μg/ml rLN-α2G. Among the anti-integrin MAbs tested, MAb 3E1, which is directed to the extracellular domain of the $\beta_4$ integrin subunit, significantly inhibited laminin-α2G-mediated M. leprae adherence to Cos-7 cells (from 330 ± 55 to 85 ± 32 bacteria per 100 cells; $p = 0.007$) (Figure 7C). Despite the expression of $\alpha_6$ integrin on Cos-7 cells, GOH3 MAb, which is known to block $\alpha_6$ integrin-mediated cell adhesion (Sonnenberg et al., 1990), had almost no effect. None of the other anti-integrin MAbs, including anti-$\beta_1$ subunit, influenced the binding even at high concentrations (Figure 7C, inset). The inhibitory action of anti-$\beta_4$ antibody suggests that the $\beta_4$ subunit of the $\alpha_6\beta_4$ integrin may be a candidate receptor mediating the M. leprae–host cell interaction through the LN-α2G domain.

## Discussion

In the present study, we provide evidence that the neural tropism of leprosy bacilli is attributable to the specific targeting of bacteria to the G domain of the LN-α2 chain expressed on the Schwann cell–axon unit. The results suggest a novel role for the G domain not only as a bacterial binding site within the endoneurial isoform of LN, but also as a tissue-restricted bridging molecule that specifically mediates M. leprae attachment to Schwann cells of the peripheral nerve. The G domain was also identified as a cell binding site of human LN-2. We propose the LN receptor $\beta_4$ integrin subunit as a candidate receptor for the LN-α2G-mediated M. leprae–cell interaction.

M. leprae preferentially binds to the LN-2 isoform, especially the α2 heavy chain. This was substantiated by several lines of evidence. Binding to α1, β1, and γ1 LN chains was excluded by the lack of M. leprae adherence to LN-1. The β2 LN chain was excluded by the binding of M. leprae to myelinating Schwann cell-neuron cultures expressing only α2, β1, and γ1 LN chains. Finally, M. leprae fails to adhere to nerve sections of LN-α2 chain-deficient *dy/dy* mice in situ. Hence, LN-α2 chain was found to be necessary and sufficient for adherence to Schwann cells. Our data also suggest that M. leprae binds to LN-α2 chain regardless of its origin, since this bacteria avidly binds to rat and murine LN-α2 in vitro and in situ, and this binding can be competitively inhibited by human LN-2/4.

The preferential binding of M. leprae to the surface of cultured Schwann cells (reviewed in Lumsden, 1959; Mukherjee and Antia, 1986) may be explained by the affinity of M. leprae for LN-α2 chain, since the binding varies in proportion to the amount of LN-α2 chain. Previous studies have also shown that M. leprae readily adheres to Schwannoma cell lines (Maeda and Narita, 1987). This can be attributed to the large amount of LN-α2 polypeptide expressed by these cells (Engvall, 1993). Conversely, although fibronectin can mediate attachment of M. leprae to epithelial and Schwannoma cell lines (Schorey et al., 1995), this mechanism is unlikely to be operative in vivo, as Schwann cells fail to express fibronectin in primary culture or in situ (Cornbrooks et al., 1983; Jaakkola et al., 1989).

Various LN isoforms differ extensively with respect to their tissue distribution in vivo (Engvall et al., 1990; Timpl and Brown, 1994). LN-2 is restricted to the basal lamina of Schwann cells, striated muscles, and trophoblast of the placenta (Leivo and Engvall, 1988; Engvall et al., 1990, Engvall, 1993). Interestingly, the presence of M. leprae in striated muscles of leprosy patients is a common phenomenon and has also been demonstrated in murine models of leprosy (Pearson et al., 1970; Esiri et al., 1972). Moreover, M. leprae invasion of the placenta in murine models of leprosy has been documented (Duncan et al., 1984) and may be responsible for the intrauterine dissemination of M. leprae (Duncan et al., 1983). It

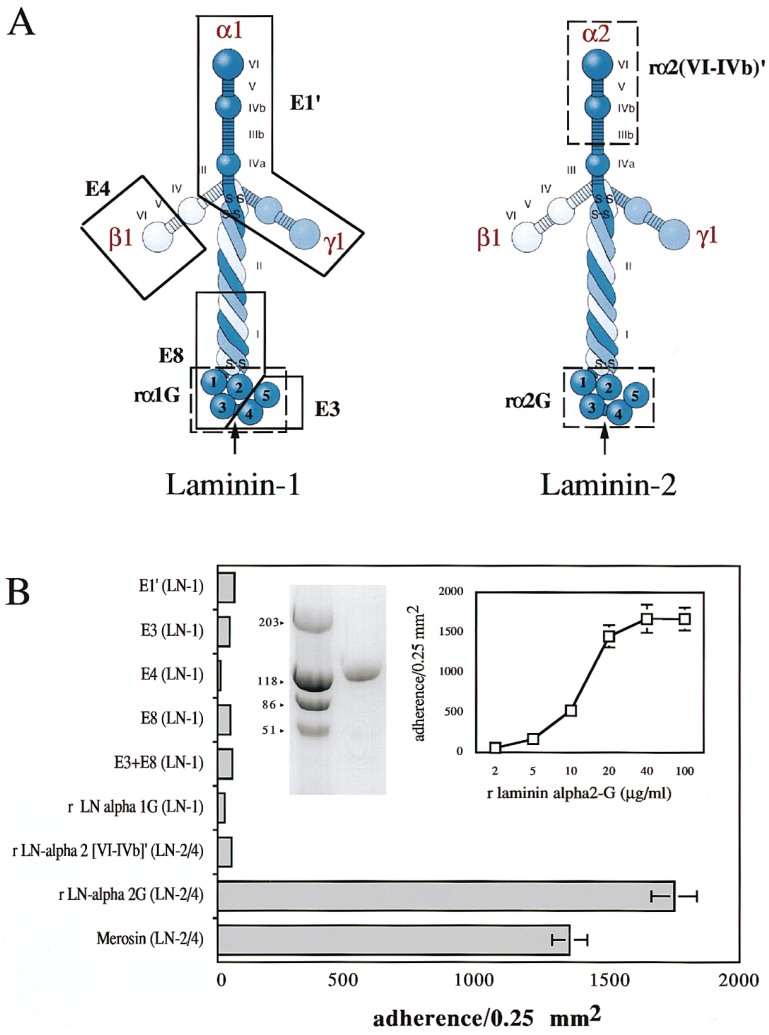


Figure 5. M. leprae Binds to the G Domain of the LN-α2 Chain

(A) Diagram of LN-1 (left) and LN-2 (right) molecules and their recombinant proteins (dotted lines) and proteolytic fragments (solid lines). Numbering and cleavage site (shown by the arrow) of the G subdomains are indicated.

(B) M. leprae binding to immobilized protein fragments of LN-1 and LN-α2, and total LN-2/4 (merosin). Left inset: Coomassie blue-stained SDS–PAGE gel showing the purity of rLN-α2G (120 kDa, right lane) and the molecular mass standard (shown in kilodaltons by the arrowheads, left lane). Right inset: the binding of M. leprae to increasing concentrations of rLN-α2G domain (mean ± SD from 3 determinations).

appears, therefore, that the tissue distribution of LN-2 correlates with sites of natural M. leprae infection. The present study provides a demonstration that a host-derived bridging molecule determines tissue-specific tropism of a bacterial pathogen.

LN-α2 chain is processed in tissues into a 300 kDa N-terminal segment and an 80 kDa C-terminal segment that includes the G domain (G2 through G5 subdomains; Figure 5A) (Leivo and Engvall, 1988). Mapping of the M. leprae-binding site using recombinant LN-α2 fragments revealed that M. leprae specifically binds to the C-terminal G domain. Lack of M. leprae binding to the analogous rLN-α1G is consistent with the particularly high sequence divergence of α1 and α2G domains (Sasaki et al., 1988; Vuolteenaho et al., 1994). The M. leprae-binding site of the α2G domain is also apparently independent of the heparin-binding subdomain (Yurchenco et al., 1993). It remains to be demonstrated directly that M. leprae binds to the proximal G1–G3 subdomains.

Exogeneous LN-2/4 competitively inhibited M. leprae binding to Schwann cell–neuron cultures characterized by the continuous presence of LN-2 on the cell surface. In forskolin-treated Schwann cells, a significant portion of LN receptors may be unoccupied, resulting in the discontinous presence of surface LN. Exogenous LN can bind these exposed receptors and thereby enhance M. leprae interactions. Similarly, rLN-α2G domain significantly increased the binding of M. leprae to forskolin-treated Schwann cells. Furthermore, although M. leprae failed to bind to *dy/dy* nerves, rLN-α2G reconstituted the binding to almost 80% of wild-type levels, suggesting that rLN-α2G directly mediates M. leprae interaction with LN receptor in *dy/dy* nerve lacking LN-α2 chain.

Study of the cell-binding activity of the G domain has been extremely difficult, since the isolated G domain does not retain activity or native conformation (Deutzmann et al., 1990; Sung et al., 1993). Consistent with this experience, rLN-α2G alone had a weak cell-binding activity (data not shown). Interestingly, in the presence of M. leprae, rLN-α2G strongly bound to primary Schwann cells and Cos-7 cells, suggesting that specific association of M. leprae and rLN-α2G alters the G domain such that it becomes competent for cell binding. These data demonstrate that the G domain, in addition to being the site for M. leprae binding, also serves as a cell-binding site of human LN-2. This system could be a useful model for the identification of cell-surface LN

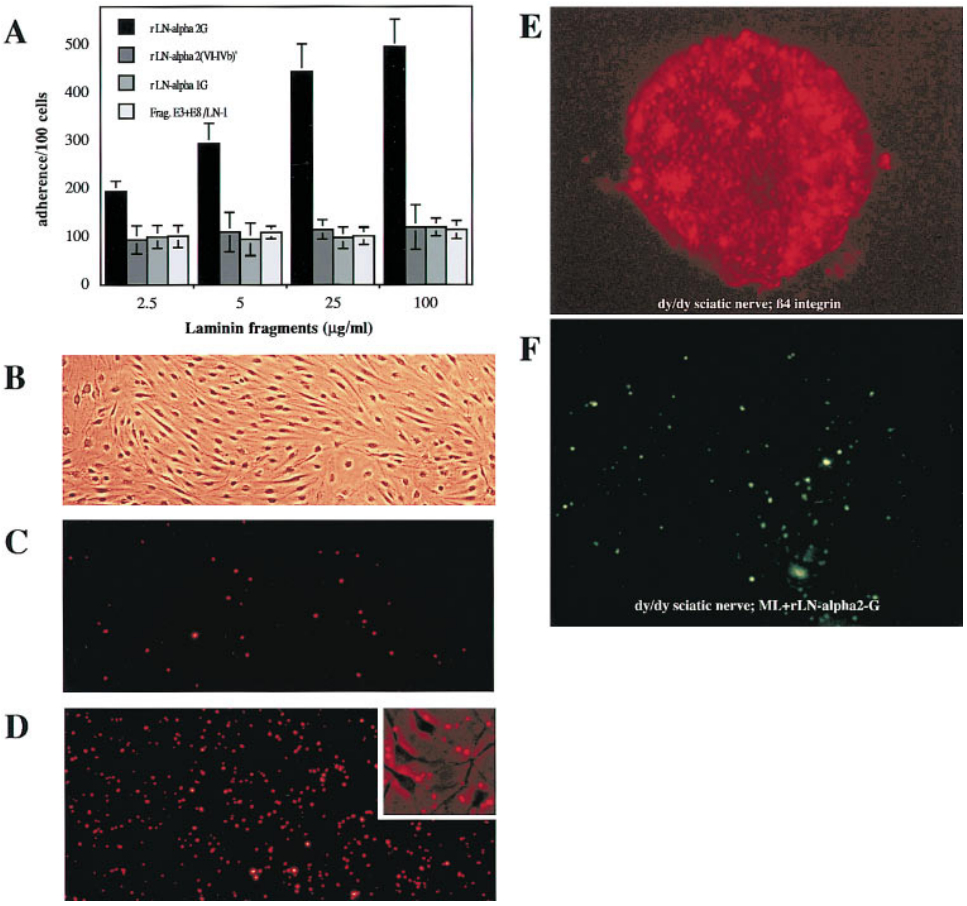


Figure 6. Role of rLN-α2G Domain in M. leprae Adherence to Schwann Cells In Vitro and In Situ

(A) M. leprae ($1 \times 10^6$) were preincubated with increasing concentrations of rLN-α2G, rLN-α2(VI–IVb)′, rLN-α1G, and E3 + E8 proteolytic fragments of LN-1 before addition to confluent cultures of forskolin-treated primary Schwann cells. Numbers of Schwann cell-bound M. leprae were counted and data were expressed as mean ± SD from 3 determinations. The experiment was repeated twice with similar results.

(B–D) M. leprae adherence to 10-day-old forskolin-treated primary Schwann cells in the presence of N-terminal r(VI–IVb)′ (C) and C-terminal rG (D) fragments (25 μg/ml) of the LN-α2 chain. Inset (D) shows higher magnification of Schwann cell-bound M. leprae. Phase-contrast image of (C) is shown in (B).

(E) In situ expression of LN receptor $\beta_4$ integrin in *dy/dy* sciatic nerve. Sections of *dy/dy* nerves were incubated with PAb to $\beta_4$ cytoplasmic domain (1:50), and bound antibody was visualized by rhodamine-labeled secondary antibody.

(F) In situ adherence to fresh-frozen sections of *dy/dy* sciatic nerve of M. leprae preincubated with excess (100 μg/ml) rLN-α2G.

receptors and for delineation of the role of LN-α2G in cell–matrix interaction in peripheral nerves and muscles. In particular, this may be of relevance to congenital muscular dystrophy and the murine *dy/dy* model, where LN-α2 plays an important role in the pathogenesis of muscle degeneration and peripheral neuropathy (Campbell, 1995).

The ability of rLN-α2G to bind efficiently to Cos-7 cells in the presence of M. leprae enabled the identification of the cell-surface integrins involved in rLN-α2G-mediated cell binding independent of the influence of other regions of the laminin molecule. Our results show that rLN-α2G-mediated M. leprae binding is partially blocked by MAb 3E1 against the $\beta_4$ integrin subunit, suggesting that cell-surface $\beta_4$ integrin may be a candidate receptor involved in the rLN-α2G-mediated attachment. Furthermore, since $\beta_4$ integrin is normally expressed in *dy/dy* nerve (Figure 6E) and M. leprae adhered to *dy/dy* nerve only in the presence of rLN-α2G, $\beta_4$ integrin may mediate adherence in situ. However, we were unable to confirm this, as no blocking antibodies are available to murine $\beta_4$. Conversely, although Cos-7 cells strongly express $\alpha_6$ integrin, the only known partner for $\beta_4$, i.e., blocking antibody GOH3 against the $\alpha_6$ subunit, showed no significant inhibitory effect on α2G-mediated M. leprae binding to Cos-7 cells. This may be due to the involvement of the nonactive site of $\alpha_6$ integrin for LN-α2G binding. It is also possible that a new integrin subunit, as yet unidentified, associated with $\beta_4$ might be involved in this interaction. Of interest, recent data suggest that α-dystroglycan may also serve as a Schwann cell receptor for LN-2 (Yamada et al., 1996). Therefore, we cannot exclude the possibility of the involvement of α-dystroglycan in M. leprae–Schwann cell interaction.

In conclusion, LN-α2G on Schwann cell basal lamina serves as a neural target of M. leprae, and the LN-α2 chain is necessary and sufficient for adherence to Schwann cells. Details of this interaction will clarify

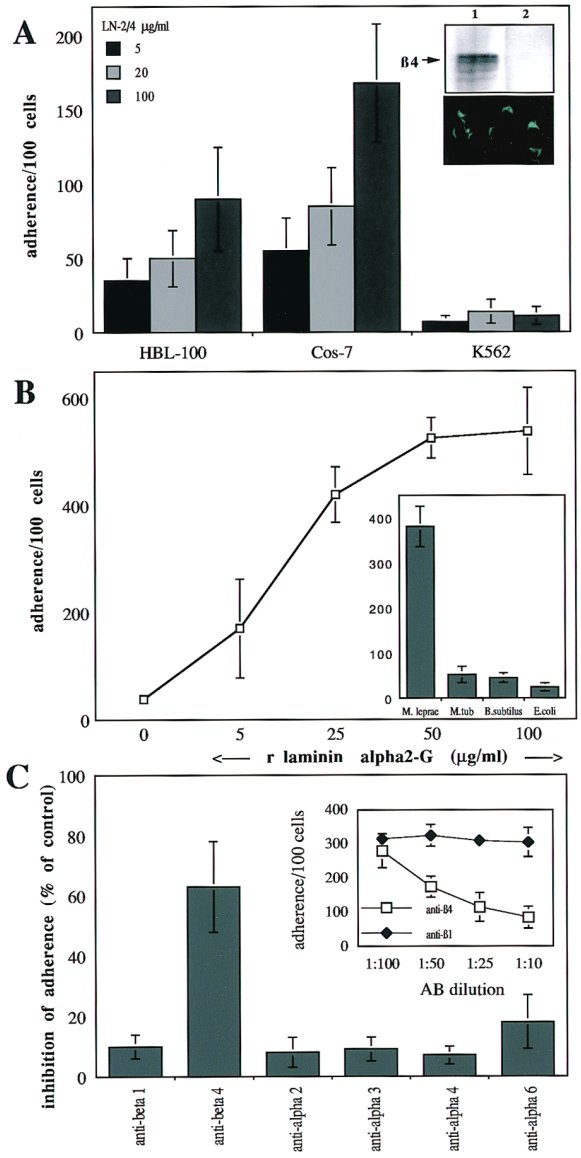


Figure 7. Involvement of LN Receptor in LN-α2G-Mediated M. leprae Adherence

(A) LN-2/4-mediated M. leprae adherence to cell lines with and without LN receptor. HBL-100, Cos-7, and K562 cell lines were inoculated with M. leprae pretreated with excess LN-2/4. Numbers of adherent M. leprae were quantified and expressed as mean ± SD per 100 cells. Insets display expression of $\beta_4$ on Cos-7 cells: above, immunoblotting of the lysates of Cos-7 (lane 1) and K562 (lane 2) using anti-$\beta_4$ MAb 3E1; below, immunofluorescence labeling of $\beta_4$ expression on Cos-7 cells as detected by MAb 3E1 (1:50) (anti-$\alpha_6$ integrin MAb also showed similar activity).

(B) M. leprae was preincubated with increasing concentrations of rLN-α2G before addition to confluent cultures of Cos-7 cells. Inset: comparison of the effect of rLN-α2G on adherence of M. leprae, M. tuberculosis, B. subtilis, and E. coli to Cos-7 cells.

(C) Effects of anti-integrin MAbs on rLN-α2G-mediated M. leprae binding to Cos-7 cells. Confluent cultures of Cos-7 cells were preincubated with indicated MAbs prior to the addition of rLN-α2G-treated M. leprae. Number of cell-bound bacteria were counted and data expressed as inhibition of adherence (percentage of control) or adherence per 100 cells (inset) from 3 determinations. The experiment was repeated twice with similar results. Inset: dilution effect of anti-$\beta_1$ and anti-$\beta_4$ MAbs on rLN-α2G-mediated M. leprae adherence to Cos-7 cells.

questions in LN matrix biology, especially those related to the G domain, using novel assays described herein. We also provide evidence that the LN receptor $\beta_4$ integrin may be involved in LN-α2G-mediated M. leprae–cell interaction. We propose that specific interaction of M. leprae with neural- and muscle-restricted LN-α2 is the basis for the neural tropism of M. leprae.

## Experimental Procedures

### Bacteria

M. leprae, isolated from four armadillos, was provided by Dr. P. J. Brennan (Colorado State University, Fort Collins, CO). M. leprae was detected either by using auramine-rhodamine Bacto TB Fluorescent Stain Kit (Difco, Detroit, MI) (Schlesinger and Horwitz, 1991), or by antibody to M. leprae-specific phenolic glycolipid (PGL-1). M. tuberculosis gamma-irradiated strains H37Rv and live avirulent H37Ra were provided by Drs. P. J. Brennan and G. Kaplan (Rockefeller University, NY), respectively. B. subtilis and E. coli strain DH5a were provided by Dr. H. R. Masure (Rockefeller University, NY).

### Antibodies

The following polyclonal (PAb) and monoclonal (MAb) antibodies to LN and LN chains were used: affinity-purified rabbit PAb to human placental LN (LN-2/4) (GIBCO–BRL, Gaithersberg, MD) and EHS LN (LN-1) (Sigma, St. Louis, MO); MAbs 2E8 and D18 to γ1/B1 LN chain; MAbs D5 and D7 to β2/S LN chain; MAb M3F7 to type IV collagen (Developmental Studies Hybridoma Bank, University of Iowa, IA, and Johns Hopkins University School of Medicine, Baltimore, MD); MAb 5H2/H6 to α2/M LN chain (Dr. E. Ingvall, La Jolla Cancer Research Foundation, La Jolla, CA); PAb 321 against 300 kDa fragment of α2 LN chain (Dr. M. Paulsson, University of Cologne, Cologne, Germany); and rabbit PAb against E3 fragment (LN-α1G4/5) (Yurchenco et al., 1993).

The following antibodies to integrin subunits were used: MAb P4C10 to $\beta_1$; MAb 3E1 to $\beta_4$ (GIBO–BRL); mouse MAbs 450–9D, 450–10D, and 450–11A, and rat MAb 439–9B to $\beta_4$ (Dr. S. J. Kennel, Oak Ridge National Laboratories, Oak Ridge, TN); rabbit PAb against synthetic peptides of carboxy-terminal sequences of $\beta_4$ (Dr. F. Giancotti, Memorial Sloan-Kettering Cancer Center, New York, NY); rat blocking MAb GOH3 to $\alpha_6$ (Dr. A. Sonnenberg, Amsterdam, The Netherlands); and blocking MAbs P1E6 to $\alpha_2$, P1B5 to $\alpha_3$, and P4G9 to $\alpha_4$ (Dako, Santa Barbara, CA).

MAb F47–21 (IgG1) to M. leprae-specific native PGL-1 was a gift from Dr. A. H. J. Kolk (Royal Tropical Institute, Amsterdam). IgG fraction of rabbit PAb to S-100 antigen and mouse IgG isotype control antibodies were from Sigma and Dako, respectively.

### LN and Other Matrix Proteins

Human placental LN, murine EHS tumor LN (LN-1), human fibronectin, heparin sulfate proteoglycan, and heparin were obtained from Sigma (St. Louis, MO). Human merosin (LN-2/4) used in this study was from GIBCO–BRL and Dr. M. Paulsson. Human type IV collagen was purchased from Collaborative Biomedical Products (Bedford, MA).

### Preparation of Recombinant Human LN-α2G Domain and Antibody

A λ-clone encoding the distal portion of the human LN-α2 chain (clone 271 kindly provided by Dr. E. Engvall) was restricted between TthIII and EcoR1 and subcloned into Blue Script KS. An AsnII–BamH1 fragment was ligated into the modified baculovirus expression vector pVL1392ss (Yurchenco et al., 1993) previously restricted with PstI and BamH1, using the complementing synthetic oligonucleotides 5′-GGTGACTGCATT-3′ and 3′-ACGTCCACTGACGTA AGC-5′ as a linker. Insect Sf9 cells were transfected and recombinant baculovirus was selected as previously described (Yurchenco et al., 1993). Recombinant glycoprotein, which was secreted, was purified from conditioned medium by heparin affinity chromatography, also as described.

A New Zealand white rabbit was immunized with purified protein

in complete Freund's adjuvant and boosted two weeks later in incomplete Freund's adjuvant. The pooled sera were purified by protein A affinity chromatography. The antibody reacted in ELISA assays and in Western blots, with the G domain of LN containing the α2 chain, but not the α1 chain. The antibody was also evaluated by immunofluorescence with frozen sections of human skeletal muscle and peripheral nerves. The antibody reacted in a restricted fashion with the perimyotubular basement membranes and Schwann cell basal lamina.

Recombinant α2(VI–IVb)′ was prepared from 293 mammalian cells transfected with pCIS-human LN α2(VI–IVb)′ cDNA (H. Colognato-Pyke and P. D. Y., unpublished data). The protein was purified from conditioned medium as described (Colognato-Pyke et al., 1995).

Preparation, purification, and characterization of recombinant LN-α1G domain have been previously described (Yurchenco et al., 1993). Proteolytic fragments of LN-1 were prepared as previously described (Yurchenco et al., 1993).

### Primary Schwann Cells and Schwann Cell–Neuron Cocultures

Cultures of primary rat Schwann cells, dorsal root ganglion (DRG) neurons, and myelinating Schwann cells–DRG neurons were established as described previously (Einheber et al., 1993). In some experiments, Schwann cells were grown on poly-L-lysine-coated Lab-tek 8-well chamber slides (Nunc Inc., Naperville, IL) in standard media and were continuously treated with a crude preparation of GGF and 2 μM forskolin.

To establish myelinating Schwann cell–neuron cocultures, DRGs were removed from E15 or E16 rats, dissociated with trypsin, and grown on collagen-coated glass coverslips in a 4-well dish (Nunc). Cultures were cycled with antimitotic agents in standard serum-containing media to remove nonneuronal cells. DRG neuron cultures were seeded with $2 \times 10^5$ Schwann cells in standard media. After 24 hr, the standard media were replaced with N2-defined media in which Schwann cells proliferate but do not assemble basal lamina or myelinate (Moya et al., 1980). After several days, media were supplemented with 50 μg/ml ascorbic acid to promote basal lamina formation and initiate myelination.

### Cell Lines

The following cell lines were used: human mammary cell line HBL-100; human erythroleukemic cell line K562 (Dr. A. Sonnenberg, The Netherlands Cancer Institute, Amsterdam); and COS-7 (ATCC CRL1651). The cells were grown in DMEM supplemented with 10% fetal bovine serum, penicillin, and streptomycin.

### Bacterial Adherence Assays

#### *LN Binding Assay*

Terasaki plates (Robbins Scientific, Sunnyvale, CA) were coated overnight at room temperature (Van der Flier et al., 1995) with 0.1 μg of LNs, type IV collagen, heparin sulfate proteoglycan, heparin, fibronectin, or recombinant and proteolytic fragments of LNs (0.25 μg). Saline or bovine serum albumin (BSA) served as negative controls. Nonspecific binding was blocked with 5% BSA for 3 hr at 37°C and then washed 5 times with DPBS. Ten microliters of bacterial suspension ($5 \times 10^8$ bacteria/ml) was added to each well and incubated for 1 hr at 37°C. Unbound bacteria were removed by washing 5 times with DPBS. After fixation with 2.5% glutaraldehyde (Sigma) for 10 min, bacteria were stained with acid-fast staining and counted visually under a fluorescence microscope. M. leprae adherence was expressed as the number of attached bacteria per 0.25 $mm^2$-calibrated grid area using 20× magnification, and net binding was calculated by subtracting the adherence to saline- or BSA-coated wells. Each experiment was repeated 3–4 times.

#### *Assay for M. leprae Adherence to Schwann Cells–Neurons*

Schwann cell–neuron cocultures grown for 8–10 weeks were inoculated with $1–5 \times 10^8$ M. leprae. After 30 min at 37°C in 5% $CO_2$, cultures were washed 5–6 times with DMEM without serum and fixed in 2.5% glutaraldehyde. M. leprae bound to Schwann cell–neuron was detected, and the number of M. leprae adherent to 100 cells (counted on the basis of nuclei) was determined visually. For competitive inhibition studies, M. leprae was preincubated with 200 μg/ml merosin (LN-2/4), type IV collagen, or fibronectin for 1 hr at 37°C before adding to the cultures. For antibody-inhibiting studies, Schwann cell–neuron cultures were incubated for 1 hr at 37°C with ~100 μg of affinity-purified antibodies to total placental LN (LN-2/4) and type IV collagen, and cultures were washed before inoculation with M. leprae. To determine M. leprae binding to primary Schwann cells, confluent cultures of forskolin-treated Schwann cells grown (10–14 days) in 8-well Lab-Tek chamber slides (Nunc) were used. To evaluate the effects of LNs and their fragments on M. leprae binding to primary Schwann cells, bacteria were preincubated with either 100 μg/ml total LN or 25 μg/ml of LN fragments for 1 hr at 37°C before inoculation.

#### *Assay of M. leprae Adherence to Heterologous Model Cell Lines*

M. leprae adherence to HBL-100, COS-7, and K562 cell lines was assayed in 8-well Lab-Tek chamber slides. M. leprae was preincubated with LN-2/4 or rLN-α2G for 1 hr at 37°C, then added to the cells for 30 min at 37°C, followed by washing. For inhibition studies, confluent cultures were incubated with antibodies against LN-receptor integrin subunits for 1 hr at 37°C before inoculation with M. leprae pretreated with LN-2/4 or rLN-α2G.

#### *In Situ Adherence Assay for M. leprae*

M. leprae adherence to peripheral nerve sections in situ was determined by adapting the protocol of Falk et al. (1993). In brief, 7–8 μm thick fresh-frozen sections of sciatic nerves from control and *dy/dy* mice were rinsed in PBS and then incubated for 1 hr in blocking buffer (0.2% BSA, 0.01% Tween-20 prepared in PBS). M. leprae suspension was diluted to $1 \times 10^6$ to $1 \times 10^8$ bacteria/ml in blocking buffer, and 100 μl was applied to the sections, which were then incubated for 30 min at 37°C. Slides were washed at least 6 times with PBS, fixed with 2.5% glutaraldehyde, and numbers of tissue-adherent bacteria per 5 randomly selected high power fields were counted. For inhibition studies, the bacterial suspension was preincubated with excess LN proteins (200 μg/ml) for 1 hr at 37°C prior to incubation with sections.

### Immunofluorescence Microscopy

Immunolabeling was performed as previously described (Rambukkana et al., 1993). In brief, slides were incubated with 2% BSA in DPBS and then incubated with primary antibodies. Slides were washed and treated with species-specific affinity-purified rhodamine- or FITC-conjugated swine anti-rabbit IgG or rhodamine- or fluorescein-conjugated rabbit anti-mouse IgG (Chemicon International, Inc., Temecula, CA). For double-labeling, cultures were incubated with the mixure of mouse MAb (IgG1) to M. leprae phenolic glycolipid-1 and rabbit antibody to LN-α2G, followed by a mixture of rhodamine-conjugated goat anti-mouse IgG and fluorescein-conjugated swine anti-rabbit IgG. All incubations were carried out at room temperature for 1 hr. Slides were washed in DPBS, mounted, and examined by epifluorescence with a Nikon microscope.

### Immunoblotting

Cell lysates of confluent cultures of primary Schwann cells, epithelial cell lines, and myelinating Schwann cell–neuron cocultures were prepared as described previously (Einheber et al., 1993). Twenty-five micrograms of protein from each lysate was fractionated on 6% and 10% SDS-polyacrylamide gels and blotted onto polyvinylidene difluoride membrane (BIO-RAD, Richmond, CA). Blots were incubated with primary antibodies followed by species-specific horseradish peroxidase-labeled affinity-purified goat anti-rabbit or goat anti-mouse antibodies (BioRad), and the reaction was developed by enhanced chemiluminescence-detection method (Amersham Intl.).

### Animals and Histological Examination of Tissues

C57BL/6 strain dystrophic homozygous *dy/dy* mice (7–8 weeks) and age-matched normal control +/+ mice were obtained from Jackson Laboratory (Bar Harbor, ME). Sciatic nerves from both control and *dy/dy* mice were removed; a portion of each was snap frozen, and the other was embedded in paraffin using routine techniques. Sections were cut and immunofluorescence with antibodies to LN-α2 chain detected essentially normal expression of LN-α2 in the nerve sections of control mice, whereas no LN-α2 was detected in *dy/dy* mice.

**Statistical Analysis**

All results were expressed as mean ± SD. Statistical analysis of the data were performed using Welch's t test for unpaired data with Instat software (Graphpad). Statistical significance was accepted for $p < 0.05$.

**Acknowledgments**

All correspondence should be addressed to A. R. We gratefully acknowledge the following contributions: M. leprae was provided by P. J. Brennan through the National Institute of Allergy and Infectious Diseases, National Institutes of Health, contract AI-52582; G. Zanazzi provided invaluable assistance with Schwann cell cultures; and M. Leismann assisted with cell line cultures. We thank T. Mathus, J. O'Rear, and S. Einheber for their contribution, and M. Zschack for graphics. We acknowledge E. Engvall, F. Giancotti, S. J. Kennel, A. H. J. Kolk, M. Paulsson, A. Sonnenberg, and J. R. Sanes for the gifts of antibodies, cell lines, and laminin reagents. We also thank M. Harboe for helpful discussion. This investigation was supported by the Heiser Foundation for Leprosy and Tuberculosis Research, a grant from the UNDP/World Bank/WHO Special Program for Research in Tropical Diseases (TDR), and by NIH grants A127913 (to E. T.), DK36425 and DK48045 (to P. D. Y.), and NS33165 (to J. L. S.), and National Multiple Sclerosis Grant RG2678 (to J. L. S.).

Received December 16, 1996; revised January 23, 1997.

**References**

Anton, E.S., Sandrock, A.W., and Matthew, W.D. (1994). Merosin promotes neurite growth and Schwann cell migration in vitro and nerve regeneration in vivo: evidence using an antibody to merosin, ARM-1. Dev. Biol. *164*, 133–146.

Bradley, W.G., and Jenkison, M. (1975). Neural abnormalities in the dystrophic mouse. J. Neurol. Sci. *25*, 249–255.

Bunge, M.B., Clark, M.B., Dean, A.C., Eldridge, C.F., and Bunge, R.P. (1990). Schwann cell function depends upon axonal signals and basal lamina components. Ann. NY Acad. Sci. *580*, 281–287.

Burgeson, R.E., Chiquet, M., Duetzmann, R., Ekblom, P., Engel, J., Kleinman, H., Martin, G., Meneguzzi, G., Paulsson, M., Sanes, J., et al. (1994). A new nomenclature for the laminins. Matrix Biol. *14*, 209–211.

Campbell, K.P. (1995). Three muscular dystrophies: loss of cytoskeleton-extra cellular matrix linkage. Cell *80*, 675–679.

Colognato-Pyke, H., O'Rear, J.J., Yamada, Y., Carbonetto, S., Cheng, Y.S., and Yurchenco, P.D. (1995). Mapping of network-forming, heparin-binding, and alpha 1 beta 1 integrin-recognition sites within the alpha-chain short arm of laminin-1. J. Biol. Chem. *270*, 9398–9406.

Cornbrooks, C.J., Carey, D.J., McDonald, J.A., Timpl, R., and Bunge, R.P. (1983). In vivo and in vitro observations on laminin production by Schwann cells. Proc. Natl. Acad. Sci. USA *80*, 3850–3854.

Danielssen, D.C., and Boeck, W. (1848). Traité dé la Spédalskhed ou Eléphantiasis des Grees (Paris: J. B. Baillére).

Delwel, G.O., Hogervorst, F., Kuikman, I., Paulsson, M., Timpl, R., and Sonnenberg, A. (1993). Expression and function of the cytoplasmic variants of the integrin alpha 6 subunit in transfected K562 cells. Activation-dependent adhesion and interaction with isoforms of laminin. J. Biol. Chem. *268*, 25865–25875.

Deutzmann, R., Aumailley, M., Wiedemann, H., Pysney, W., Timpl, R., and Edgar, D. (1990). Cell adhesion, spreading and neurite stimulation by laminin fragment E8 depends on maintenance of secondary and tertiary structure in its rod and globular domain. Eur. J. Biochem. *191*, 513–522.

Duncan, M.E., Melsom, R., Pearson, J.M., Menzel, S., and Barnetson, R.S. (1983). A clinical and immunological study of four babies of mothers with lepromatous leprosy, two of whom developed leprosy in infancy. Int. J. Leprosy *51*, 7–17.

Duncan, M.E., Fox, H., Harkness, R.A., and Rees, R.J. (1984). The placenta in leprosy. Placenta *5*, 189–198.

Einheber, S., Milner, T.A., Giancotti, F., and Salzer, J.L. (1993). Axonal regulation of Schwann cell integrin expression suggests a role for alpha 6 beta 4 in myelination. J. Cell Biol. *123*, 1223–1236.

Einheber, S., Hannocks, M.J., Metz, C.N., Rifkin, D.B., and Salzer, J.L. (1995). Transforming growth factor-beta 1 regulates axon/Schwann cell interactions. J. Cell Biol. *129*, 443–458.

Engvall, E. (1993). Laminin varients: why, where and when? Kidney Int. *43*, 2–6.

Engvall, E., and Wewer, U.M. (1996). Domains of laminin. J. Cell. Biochem. *61*, 493–501.

Engvall, E., Earwicker, D., Haaparanta, T., Ruoslahti, E., and Sanes, J.R. (1990). Distribution and isolation of four laminin varients; restricted tissue distribution of heterotrimers assembled from five different subunits. Cell Regul. *1*, 731–740.

Engvall, E., Earwicker, D., Day, A., Muir, D., Manthorpe, M., and Paulsson, M. (1992). Merosin promotes cell attachment and neurite outgrowth and is a component of the neurite-promoting factor of RN22 Schwannoma cells. Exp. Cell Res. *198*, 115–123.

Esiri, M.M., Weddell, A.G., and Rees, R.J. (1972). Infection of murine striated muscle with Mycobacterium leprae: a study by light and electron microscopy. J. Pathol. *106*, 73–80.

Falk, P., Roth, K.A., Boren, T., Westblom, T.U., Gordon, J.I., and Normak, S. (1993). An in situ adherence assay reveals that Helicobacter pylori exhibits cell lineage-specific tropism in the human gastric epithelium. Proc. Natl. Acad. Sci. USA *90*, 2035–2039.

Falkow, S. (1991). Bacterial entry into eukaryotic cells. Cell *65*, 1099–1102.

Feltri, M.L., Scherer, S.S., Nemni, R., Kamholz, J., Vogelbacker, H., Scott, M.O., Canal, N., Quaranta, V., and Wrabetz, L. (1994). Beta 4 integrin expression in myelinating Schwann cells is polarized, developmentally regulated and axonally dependent. Development *120*, 1287–1301.

Gamble, H.J. and Eames, R.A. (1964). Electron microscope study of the connective tissue of human peripheral nerve. J. Anat. *98*, 655–663.

Hoepelmann, A.I.M., and Tuomanen, E.I. (1992). The consequences of microbial attachment: directing host cell functions with adhesins. Infect. Immun. *60*, 1729–1733.

Hynes, R.O. (1992). Integrins, versatility, modulation, and signaling in cell adhesion. Cell *69*, 11–25.

Isberg, R.R., and Leong, J.M. (1990). Multiple beta 1 chain integrins are receptors for invasin, a protein that promotes bacterial penetration into mammalian cells. Cell *60*, 861–871.

Jaakkola, S., Peltonen, J., Riccardi, V., Chu, M.L., and Uitto, J. (1989). Type 1 neurofibromatosis: selective expression of extracellular matrix genes by Schwann cells, perineurial cells, and fibroblasts in mixed cultures. J. Clin. Invest. *84*, 253–261.

Jaakkola, S., Savunen, O., Halme, T., Uitto, J., and Peltonen, J. (1993). Basement membranes during development of human nerve: Schwann cells and perineurial cells display marked changes in their expression profiles for laminin subunits and beta 1 and beta 4 integrins. J. Neurocytol. *22*, 215–230.

Jessen, K.R., and Mirsky, R. (1991). Schwann cell precursors and their development. Glia *4*, 185–194.

Job, C.K. (1989). Nerve damage in leprosy. Int. J. Leprosy *57*, 532–539.

Leivo, I., and Engvall, E. (1988). Merosin, a protein specific for basement membranes of Schwann cells, striated muscle, and trophoblast, is expressed late in nerve and muscle development. Proc. Natl. Acad. Sci. USA *85*, 1544–1548.

Lumsden, C.E. (1959). Leprosy and the Schwann cell in vitro and in vivo. In Leprosy in Theory and Practice, R.D. Cochrane, ed. (Bristol, UK: John Wright and Sons, Ltd.), pp. 221–250.

Maeda, M., and Narita, M. (1987). Affinity of Mycobacterium leprae with Lewis rat Schwannoma cell line (Lewis TC 98). Lepr. Rev. *58*, 39–51.

Mercurio, A.M. (1995). Laminin receptors: achieving specificity through cooperation. Trends Cell Biol. *5*, 419–423.

Moya, F., Bunge, M.B., and Bunge, R.P. (1980). Schwann cells proliferate but fail to differentiate in defined media. Proc. Natl. Acad. Sci. USA *77*, 6902–6906.

Mukherjee, R., and Antia, N.H. (1986). Host-parasite interrelationship between M. leprae and Schwann cells in vitro. Int. J. Lepr. *54*, 632–638.

Niessen, C.M., Cremona, O., Daams, H., Ferraresi, S., Sonnenberg, A., and Marchisio, P.C. (1994). Expression of the integrin alpha 6 beta 4 in peripheral nerves: localization in Schwann and perineural cells and different variants of the beta 4 subunit. J. Cell Sci. *107*, 543–552.

Noordeen, S.K., Lopez, B.L., and Sundaresan, T.K. (1992). Estimated number of leprosy cases in the world. Bull. World Health Org. *70*, 7–10.

Pearson, J.M., Rees, R.J., and Weddell, A.G. (1970). Mycobacterium leprae in the striated muscle of patients with leprosy. Lepr. Rev. *41*, 155–166.

Relman, D., Tuomanen, E., Falkow, S., Golenbock, D.T., Saukkonen, K., and Wright, S.D. (1990). Recognition of a bacterial adhesion by an integrin: macrophage CR3 (alpha M beta 2, CD11b/CD18) binds filamentous hemagglutinin of Bordetella pertussis. Cell *61*, 1375–1382.

Rambukkana, A., Burggraaf, J.D., Faber, W.R., Harboe, M., Teeling, P., Krieg, S., and Das, P.K. (1993). The major secreted antigen 85 complex possesses epitopes that are differentially expressed in human leprosy lesions and Mycobacterium leprae infected armadillo tissues. Infect. Immun. *61*, 1835–1845.

Sanes, J.R., Engvall, E., Butkowsky, R., and Hunter, D.D. (1990). Molecular heterogeity of basal laminae: isoforms of laminin and collagen-IV at neuromuscular junction and elsewhere. J. Cell Biol. *111*, 1685–1699.

Sasaki, M., Kleinman, H.K., Huber, H., Deutzmann, R., and Yamada, Y. (1988). Laminin, a multidomain protein. The A chain has a unique globular domain and homology with the basement membrane proteoglycan and the laminin B chains. J. Cell Biol. *263*, 16536–16544.

Schlesinger, L.S., and Horwitz, M. (1991). Phagocytosis of Mycobacterium leprae by human monocyte-derived macrophages is mediated by complement receptors CR1 (CD15), CR3 (CD11b/CD18), and CR4 (CD11c/CD18) and IFN-gamma activation inhibits complement receptor function and phagocytosis of this bacterium. J. Immunol. *147*, 1983–1994.

Schorey, J.S., Li, Q., McCourt, D.W., Bong-Mastek, M., Clark-Curtiss, J.E., Ratliff, T.L., and Brown, E.J. (1995). A Mycobacterium leprae gene encoding a fibronectin binding protein is used for efficient invasion of epithelial cells and Schwann cells. Infect. Immun. *63*, 2652–2657.

Sonnenberg, A., Linders, C.T.J., Daams, J.H., and Kennel, S.J. (1990). The alpha 6 beta 1 (VLA-6) and alpha 6 beta 4 protein complexes: tissue distribution and biochemical properties. J. Cell Sci. *96*, 207–211.

Sunada, Y., Bernier, S.M., Kozak, C.A., Yamada, Y., and Campbell, K.P. (1994). Deficiency of merosin in dystrophic dy mice and genetic linkage of laminin M chain gene to dy locus. J. Biol. Chem. *269*, 13729–13732.

Sung, U., O'Rear, J.J., and Yurchenco, P.D. (1993). Cell and heparin binding in the distal long arm of laminin: identification of active and cryptic sites with recombinant and hybrid glycoprotein. J. Biol. Chem. *123*, 1255–1268.

Timpl, R., and Brown, J.C. (1994). The laminins. Matrix Biol. *14*, 275–281.

Tuomanen, E.I., Austrian, R., and Masure, H. (1995). The pathophysiology of pneumococcal infection. N. Engl. J. Med. *332*, 1280–1284.

Van der Flier, M., Chhun, N., Wizemann, T.M., Min, J., McCarthy, J.B., and Tuomanen, E.I. (1995). Adherence of streptococcus pneumoniae to immobilized fibronectin. Infect. Immun. *63*, 4317–4322.

Vuolteenaho, R., Nissinen, M., Sainio, K., Byers, M., Eddy, R., Hirvonen, H., Shows, T.B., Sariola, H., Engvall, E., and Tryggvason, K. (1994) Human laminin M chain (merosin): complete primary structure, chromosomal assignment, and expression of the M and A chain in human fetal tissues. J. Cell Biol. *124*, 381–394.

Xu, H., Christmas, P., Wu, X.-R., Wewer, U.M., and Engvall, E. (1994). Defective muscle basement membrane and lack of M-laminin in dystrophic dy/dy mouse. Proc. Natl. Acad. Sci. USA *91*, 5572–5576.

Yamada, H., Denzer, A.J., Hori, H., Tanaka, T., Anderson, L.V.B., Fujita, S., Fukuta-Ohi, H., Shimizu, T., Ruegg, M.A., and Mitsumara, K. (1996). Dystroglycan is a dual receptor for agrin and laminin-2 in Schwann cell membrane. J. Biol. Chem. *271*, 23418–23423.

Yurchenco, P.D., Cheng, Y.S., and Colognato, H. (1992). Laminin forms an independent network in basement membranes. J. Cell Biol. *117*, 1119–1133. Erratum: J. Cell Biol. *118*, 493.

Yurchenco, P.D., Sung, U., Ward, M.D., Yamada, Y., and O'Rear, J.J. (1993). Recombinant laminin G domain mediates myoblast adhesion and heparin binding. J. Biol. Chem. *268*, 8356–8365.